Nanoparticles in Daily Life: Applications, Toxicity and Regulations

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Abstract

At nanoscale, man-made materials may show unique properties that differ from bulk and dissolved counterparts. The unique properties of engineered nanomaterials not only impart critical advantages but also confer toxicity because of their unwanted interactions with different biological compartments and cellular processes. In this review, we discuss various entry routes of nanomaterials in the human body, their applications in daily life, and the mechanisms underlying their toxicity. We further explore the passage of nanomaterials into air, water, and soil ecosystems, resulting in diverse environmental impacts. Briefly, we probe the available strategies for risk assessment and risk management to assist in reducing the occupational risks of potentially hazardous engineered nanomaterials including the control banding (CB) approach. Moreover, we substantiate the need for uniform guidelines for systematic analysis of nanomaterial toxicity, *in silico* toxicological investigations, and obligation to ensure the safe disposal of nanowaste to reduce or eliminate untoward environmental and health impacts. At the end, we scrutinize global regulatory trends, hurdles, and efforts to develop better regulatory sciences in the field of nanomedicines.

Keywords

engineered nanoparticles; entry routes; environmental impact; nanowaste; disposal; control banding

I. INTRODUCTION

Nano is an umbrella term encompassing several technical and scientific fields, processes, and properties at the nanoscale or microscale.[1] The International Organization for Standardization (ISO) defines a nanoparticle as a nano-object with all three external dimensions in the nanoscale, which is approximately 1 to 100 nm.[2] Although nanoparticles have existed in the environment throughout the history of the earth (e.g., as minerals, clays, and products of bacteria) and have been intentionally used for centuries (e.g., as finely divided metal colorants), the systematically designed nanoscale materials, also called engineered nanoparticles, have only appeared in the last few decades. The unique size-

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dependent physicochemical properties of nanoparticles often promote their application in many products; however, these same unique properties also lead to unique physiological responses in living systems by interaction with these materials. Engineered nanoparticles can be more toxic than larger particles because they can move more freely than bulkier molecules.

A continuously increasing number of commercial products containing nanoparticles are available; however, only a few materials are currently used in large amounts. Therefore, they slip into our daily lives without our awareness, primarily in the form of personal care commercial products[3] (Fig. 1). Altered new properties of these nanoparticles including color, transparency, solubility, and chemical reactivity, which make them attractive candidates in the cosmetics and personal care industries.[4] They may facilitate skin absorption by promoting diffusion from the cosmetic vehicles into the surface layer of skin.[5] However, insoluble and stable nanoparticles, such as titanium dioxide, gold nanoparticles, silver nanoparticles, and polymers may enter the body and cause safety issues directly.[6]

To address occupational safety and health concerns associated with engineered nanomaterials, the National Institute for Occupational Safety and Health (NIOSH) established the Nanotechnology Research Center (NTRC) in 2004.[7] Once in the environment, nanomaterials may potentially interact with metabolic networks and cellular constituents. Considering the complexities of natural ecosystems, proper precautions must be taken while exploring the effects of these nanoparticles on terrestrial and aquatic ecosystems and establishing their environmental relevance.[8] Generally, nanomaterial waste products are disposed in a similar manner to conventional wastes, without any special precautions or treatment. These nanowastes could be extraordinarily hazardous and/or chemically reactive, so they should be neutralized before disposal. Governments should act proactively and develop robust nanowaste management strategies to prevent longterm unintended consequences, and, where possible, recycle these materials.[9] In this review, we discuss various exogenous and endogenous entry routes of engineered nanoparticles, the most frequently encountered nanomaterials in our daily life and their toxicities. We also discuss their environmental impacts, risk assessments, and control banding. We are herein calling for more careful handling and manipulation of engineered nanoparticles, as well as more validation and standardization of nanoparticle toxicity tests, since strict regulation can promote safe applications of nanoparticles in daily life.

II. VARIOUS EXOGENOUS AND ENDOGENOUS ENTRY ROUTES

The exogenous ingestion of engineered nanoparticles primarily results from hand-to-mouth contact in the workplace, among factory workers, engineers, and scientists working on cutting-edge products in laboratories. Alternatively, these nanoparticles can be ingested directly via food, drinking water, drugs, or drug delivery systems. In addition, nanoparticles cleared from the respiratory tract via the mucociliary escalator can subsequently travel into the gastrointestinal (GI) tract.[10] Inhalation of airborne nanoparticles is another important entry route into the human body.[11] Larger particles usually are deposited in the nasopharyngeal region (5–30 μm) by the inertial impaction mechanism, whereas smaller particles (1–5 μm) that fail to be captured in the nasopharyngeal region are deposited in the

tracheobronchial region, mainly through sedimentation. The particles may be further absorbed or removed by mucociliary clearance. Finally, the remaining submicron particles (< 1 μm) and nanoparticles (< 100 nm) with the smallest size distribution penetrate deeply into the alveolar region, where removal mechanisms may be insufficient. The deeper the particles are deposited, the longer it takes to remove them from the lung and the higher the probability of adverse health effects due to particle tissue and particle–cell interactions.[12,13] Nanosized particles are able to effectively access the alveolar region of the lungs and come into close contact with the alveolar epithelium. Once deposited, these very small particles are able to cross the blood–air–tissue barrier and enter the bloodstream, where they may readily reach other target organs.[11]

In addition, insoluble particles may remain in the lung indefinitely.[14,15] Prolonged residence of particles in the lung may lead to injury and biological responses.[16] It is also possible that inhaled ultrafine particles (UFPs), by virtue of their extremely small size, may deposit in the olfactory mucosa and then translocate in the central nervous system (CNS), which in turn might cause neurotoxicity. Recent studies demonstrate that the CNS may be a crucial target for nanoparticle inhalation or intranasal instillation exposure.[17,18] Exposure to nanoparticles is associated with a range of acute and chronic effects ranging from inflammation, exacerbation of asthma, and metal fume fever to fibrosis, chronic inflammatory lung diseases, and carcinogenesis.[19–21] Various studies have demonstrated that inhaled or injected nanoparticles could enter systemic circulation and migrate to different organs and tissues. [22,23]

III.   NANOMATERIALS IN DAILY LIFE AND THEIR TOXICITY

The most frequently encountered nanomaterials in our daily life include zinc oxide nanoparticles (ZnONPs), titanium dioxide nanoparticles (TiO$_2$NPs), silica nanoparticles (SiO$_2$NPs), silver nanoparticles (Ag-NPs), gold NPs (AuNPs), and polymeric nanoparticles (PNPs). At the target sites, different mechanisms may be responsible for the biological effects of nanoparticles. The various mechanisms responsible for nanoparticle toxicity are summarized in Table 1. Table 2 includes some detailed toxicological studies on various nanomaterials.

A.   ZnO Nanoparticles

Zinc oxide nanoparticles (ZnONPs) are prevalent in sunscreens, food additives, pigments, and biosensors. Several researchers have studied the toxic effects of these engineered ZnONPs in different cell lines and animal models. The cytotoxicity and genotoxicity potential of ZnONPs has been shown both *in vitro* and *in vivo*.[24,25] In further studies, ZnONPs have reduced cell viability in dose-dependent and time-dependent manner.[26] ZnONPs are suspected of increasing the expression of the metallothionein gene, which is considered a biomarker in metal-induced toxicity.[27] Studies have confirmed the dose-dependent hepatotoxicity and significant increase in oxidative stress through an increase in malondial- dehyde (MDA) content and decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes activity in the liver. ZnONPs also elevate plasma

aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels.

B. TiO$_2$ Nanoparticles

TiO$_2$ is extensively used as a pigment, a thickener, and a UV absorber in cosmetic and skin care products. TiO$_2$ allows osseointegration of artificial medical implants and bone. Several attempts have been made to understand the toxicity of TiO$_2$NPs. Some research showed skin penetration and toxicity of TiO$_2$NPs in hairless mice and porcine skin after subchronic dermal exposure.[28] However, most researchers believe that TiO$_2$NPs from sunscreens did not pose a significant health threat because they do not appear to significantly penetrate the skin.[29,30] Some studies have demonstrated *in vitro* cytotoxicity and genotoxicity of TiO$_2$NPs in various cell lines, plants, and brains of mice after oral administration.[31–33] Bioaccumulation, subacute toxicity, and tissue distribution of TiO$_2$NPs was exhibited in goldfish (*Carassius auratus*) and C57BL/6 mice.[34,35]

C. Silica Nanoparticles

Synthetic amorphous silica has been used as a common food additive for several decades. It is widely applied to processed foods and registered by the European Union as a food additive with the code E551,[36] The main purpose of SiO$_2$NP in the food industry is to prevent poor flow or "caking/" particularly in powdered products. SiO$_2$NPs are additionally employed as a thickener in pastes or as a carrier of flavors, and also to clarify beverages and control foaming.[37,39] Scientists have evaluated the toxicity of SiO$_2$NP as a food additive on gastrointestinal cells, indicating their safety as food additive, but suggested the necessity of long-term *in vivo* studies to confirm their safety profile.[40] Recently, researchers have reported that interactions between food additive SiO$_2$NPs and food matrices were highly dependent on the type of food component.[41] Moreover, the experimenters have suggested that toxicity of SiO$_2$NPs depends on size, dose, and cell type.[42] Researchers have also demonstrated that the shape affected biodistribution, excretion, and toxicity of mesoporous SiO$_2$NPs after oral and intravenous administration.[43,44]

D. Silver Nanoparticles

AgNPs are good antibacterial and antiviral agents and have been used to treat infection in bums, open wounds, chronic ulcers, trophic sores, eczema, and acne.[45] Likewise, the use of AgNPs as an antimicrobial agent in toothpastes, shampoos, air sanitizer sprays, detergents, and soaps has been also been reported. AgNPs have also been extensively used for packaging and storage of food products to increase their shelf life. Silver-based resin composites have been used to fill and coat dental and medical devices. Experiments have suggested that AgNP could be used as a safe preservative in cosmetics; however, when the barrier function of human skin is disrupted, they may penetrate the skin.[46] Investigators have affirmed the noncytotoxicity of ammonia and PVPs stabilized AgNPs at lower concentrations in mice.[47] Nonetheless, AgNPs damaged DNA and caused *in vitro* toxicity and functional impairment in human cell lines in other studies.[48,49] Some researchers studied the short- and long-term effects of AgNPs on human microvascular endothelial cells and suggested that their cytotoxicity and genotoxicity makes them a useful tool to control excessive angiogenesis.[50]

E. Gold Nanoparticles

The biological application of AuNPs is based on capping with biofunctional moieties possessing some significant biological activity (peptides, carbohydrates) to control cellular processes. The drug delivery applications and photothermal therapy of AuNPs are being widely explored.[51,52] Furthermore, coadministration of drugs and AuNPs can enhance therapeutic efficacy.[53] The diagnostic application of AuNPs is justified by their light reflecting ability and surface plasmon resonance phenomenon.[54] Surface functionalization of AuNPs and their ability to bind with thiols and amine groups have been used for developing AuNPs as a vector for various drugs and biological molecules.[55] Because of their enormous therapeutic and diagnostic potentials, AuNP are extensively being investigated to understand their toxicity profiles. Researchers have documented the toxicity of AuNPs on immune dendritic cells extracted from bone marrow of mice.[56] Further studies have showed that the genotoxicity of citrate-coated AuNPs in human HepG2 cells.[57] While analyzing the effect of surface coating on the biodistribution profile, up to 86% peptide-capped AuNPs were found in the rat liver.[58] Investigations to deduce the shape effect in cellular uptake of PE-Gylated AuNPs revealed that the endocytosis rate was highest for spherical AuNPs. The fastest internalization rate of spherical AuNP was followed by cubic, rodlike, and then disklike particles.[59] In addition, PEG-coated AuNPs have shown size-dependent accumulation in different organs, but their *in vivo* toxicity was not size-dependent.[60] Studies also revealed that positively charged AuNPs exerted greater influence on cellular toxicity because they were more easily transported through a negatively charged cell membrane.[61]

F. Polymeric Nanoparticles In Drug Delivery

Biodegradable polymers are the prevailing carriers for targeted and controlled drug delivery systems. However, a clear understanding of the interactions between biological systems and those PNPs is still unexplored. PNPs with a size ranging from 10 to 200 nm not only escape renal filtration and biliary excretion but also accumulate in tumors, using enhanced permeability and retention (EPR) effects. Particles larger than 200 nm undergo rapid hepatic clearance and reticuloendothelial system (RES) recognition.[62] PEGylation is a popular method to prevent PNP clearance so it remains in systemic circulation for a longer period. After *in vivo* administration of cationic PNPs, nonspecific interactions may occur with nonspecific cells or opsonizing proteins in the blood compartment because of electrostatic binding, which may cause unexpected cytotoxicity. These nonspecific surface reactivity or interactions could be minimized by employing small and relatively less negatively charged anionic (almost neutral) PNPs for a broad-spectrum biological effect. Different biodegradable polymers have been investigated for their safety when used as nanoparticles. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles were found to be safe on the bronchial epithelium, independent of their surface charge.[63] However, some researchers demonstrated surface coating (chitosan [CS]; poloxamer 188 [PF68]; poly(vinyl alcohol) [PVA]) mediated toxicity of polymeric PLGA-NP towards humanlike macrophages.[64] Zebrafish embryos treated with CS NP exhibited decreased hatching rate and concentration-dependent mortality.[65] Double emulsion technique using polymer eudragit®RS+ (DE/RS+) and double emulsion technique using polymer poly(ε-caprolactone)+ (DE/PCL+) were considered a satisfactory nano-sized delivery system for low molecular weight heparin (LMWH) because of their high encapsulation efficiency and low toxicity.[66] Poly(ε-caprolactone) (PCL)

nanoparticles loaded with oxorubicin (DOX) were biocompatible, and they enhanced the antitumor effect of DOX while reducing its toxicity in breast and lung cancer cell lines from both humans and mice.[67] The detailed toxicological studies about engineered nanoparticles are summarized in Table 2.

IV.   ENVIRONMENTAL IMPACT OF NANOPARTICLES

The nanoparticles could be released into the environment as industrial waste, directly into the air, water, and soil systems, or through remediation of contaminated lands. The journey and fate of nanoparticles in water, air, and soil is depicted in Fig. 2.

A.   Nanoparticles in Aquatic Systems

Nanoparticles may invade aquatic systems directly, in industrial discharges or wastewater treatment effluents, or indirectly, through surface runoff from soils. The dissolution of nanoparticles may release potentially toxic components into the environment. Sometimes these nanoparticles can conglomerate with coexisting nanoparticles (homoaggregates) or combine with other organic colloids/natural minerals (heteroaggregation) to significantly alter their interactions with biota and potential toxicity in environment. Aquatic nanomaterials accumulate in bottom sediments, mainly by heteroaggregation because homoaggregates tend to sediment more slowly.[68-70] Natural organic matters can modify the toxicity of metallic nanoparticles (MNPs) by remodeling several of their properties, such as suspension stabilization, bioavailability of metal ions from MNPs, electrostatic interactions and steric repulsion between MNPs and organisms, and MNP-induced generation of reactive oxygen species. The toxicity of nanomaterials to aquatic biota involves adsorption to cell surfaces and disruption to membrane transport.[71]

B.   Nanoparticles in Air

Nanoparticles suspended in the air can spread over long distances from the point of their release, resulting in uncontrollable human exposure as well as ecotoxicological effects on aquatic or terrestrial biota. The nanoparticles released into terrestrial environments are less likely to spread because of their immobility, but they can enter the human body through swallowing or direct skin contact. While dispersed in the environment, nanoparticles can undergo several potential transformations, such as dissolution, aggregation, or other reactions with biomacromolecules, depending on the properties of both the nanoparticles and the receiving medium.[72,73]

C.   Nanoparticles in Soil Systems

Nanoparticles can penetrate soils directly through fertilizers or plant protection products, or indirectly through application to land or wastewater treatment products, such as sludges or biosolids. These nanoparticles can bioaccumulate, trophically transfer, and even biomagnify in some systems, causing numerous toxic effects on soil organisms. Moreover, their untoward effects on plant-fungi and plant-bacteria have already been reported; further research on other possible interactions (e.g., competition, predation) is needed to assess potential risks. Negative effects of nanoparticles on nitrogen and other biogeochemical cycles have been shown in numerous studies.[74–77]

V. RISK ASSESSMENT AND CONTROL BANDING

The European Center for Ecotoxicology and Toxicology of Chemicals (*ECETOC*) Task Force on Nanomaterials has put forward a comprehensive functionality-driven concept for the grouping of nanomaterials.[78] This decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) consists of three tiers to assign nanomaterials to four main groups (MGs), encompassing soluble nanomaterials (MG1s), biopersistent high aspect ratio nanomaterials (MG2s), passive nanomaterials (MG3s), and active nanomaterials (MG4s). Focusing on all relevant aspects of a nanomaterial's life cycle and biological pathway, the essential grouping criteria include intrinsic material properties in Tier 1 (water solubility, particle morphology, and chemical composition) and system-dependent properties in Tier 2 (dissolution in biological media, surface reactivity, particle dispersibility, and *in vitro* effects). In Tier 3, the Tier 1 and Tier 2 MG assignment that is based on nonanimal testing alone is confirmed or corrected using data from short-term *in vivo* studies. The "rat short-term inhalation study" should be done for the inhalation route of entry, the predominant exposure route for most nanomaterials.[79,81] The DF4nanoGrouping ensures that sufficient data are available to assess the risk of a nanomaterial, and it fosters the use of nonanimal methods to save both animals and resources.[82]

Control banding (CB) can be described as a qualitative or semiquantitative approach for risk assessment and risk management to assist in reducing worker exposure to potentially hazardous engineered nanomaterials. Some CB strategies focus on assigning a specific control band based on the possible *hazard severity* of nanomaterials (e.g., toxicity indicators) or *exposure potential* (e.g., quantity used, volatility, dustiness), whereas other strategies may directly assign exposure control options based on the task performed without prior assessment of potential exposure. CB strategies include a hierarchy of risk management approaches for controlling exposures to hazardous nanomaterials that typically encompass regulation of the potential hazard, engineering controls (e.g., local exhaust ventilation, high-efficiency particulate air [HEPA] filters), good occupational hygiene practices (e.g., personal protective equipment), and the need to seek specialist advice depending on a particular CB strategy.[83,84]

A. Toxicological Assays

Before being considered for human application, all engineered nanomaterials must be subjected to toxicological studies. However, the toxicological data derived up to now are conflicting and inconsistent. Umair[85] stressed the need to follow uniform guidelines for test procedures used to systematically analyze toxicity of different nanomaterials. For toxicological investigations of nanoparticles, a number of parameters must be considered, including particle size, size distribution, surface area, surface reactivity, particle morphology, particle agglomeration, solubility, chemical composition, particle number, and mass concentrations.[13,19,86] Currently, the toxicity of engineered nanoparticles is assessed with a number of approaches. Among them, cell viability is determined by tetrazolium reduction assays; cell membrane integrity is appraised by LDH (lactate dehydrogenase) assay; apoptosis is characterized with immunohistochemistry biomarkers; the comet assay is used to analyze genotoxicity; and electron microscopy is used to visualize intracellular

localization of nanoparticles.[87,88] Furthermore, compounds such as MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium]; XTT[2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium, monosodium salt]; WST-l,2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-2H-tetrazolium; and monosodium salt are used to detect viable cells. Among all of these, the MTT tetrazolium assay has been widely adopted in laboratories for evaluation of cell toxicity.[89,90] The enzyme-linked immunosorbent assay (ELISA) is used to detect inflammatory biomarkers in cell culture. To estimate cell inflammation, chemokines IL-8, TNF-α, and IL-6 are used as biomarkers.[91,92]

B. *In Silico* Toxicology

The field of *in silico* toxicology has been evolving rapidly, as demonstrated by the introduction of new methods and improvement of the existing ones. In silico toxicology refers to the use of computational methods to analyze, simulate, visualize, or predict the toxicity of chemicals and nanomaterials. These methods complement existing toxicity tests and minimize late-stage failures in drug design. There are various methods for generating models to predict toxicity endpoints, including structural alerts (SAs) and rule-based models; read-across (RA), dose–response (DR), and time–response (TR) models; pharmacokinetic (PK) and pharmacodynamic (PD) models; uncertainty factors (UFs) models; and the quantitative structure–activity relationship (QSAR) model.[93–95]

C. Nanowaste Management

The burgeoning applications of nanotechnology result in the generation of waste containing synthetic (or engineered) nanomaterials. This so-called nanowaste is hard to monitor due to its nanoscale dimensions. It is crucial to ensure that the disposal of such waste does not stir up inimical effects on health or environment.

D. Disposal Pathways

Concentrated industrial nano waste should be diluted and deactivated before disposal. Depending on the type of the material, thermal, chemical, physical, or biochemical processing of nanotechnology-containing waste is possible to deactivate them. At present three pathways exist for disposal of this nanowaste: landfill, incineration (thermal treatment), and recycling (material recovery). The recycling process depends on the material type, and hence the conditions are different to which the product matrices containing nanomaterials are exposed. Further, recycling could impose three types of detrimental effects: (1) occupational health effects of recycling processes themselves; (2) environmental impacts related to the treatment of residue from the recycling processes, which will end up either in incineration, landfill, or sewage treatment; and (3) introduction of residual nanomaterials into recycled products.[9] Figure 3 displays various nanoparticle disposal pathways.

F. Global Regulatory Trends

In general, the strategies used for conventional drug products have been adapted to evaluate the safety/toxicity and biocompatibility of nanomedicines.[96,97] But the main difficulty underlying the regulation of nanomedicines is the evolution of sensitive assays that not only detect the low concentrations of nanomaterials but also distinguish them from metabolized forms or formed aggregates.[98] At nanoscale, aggregation is not so easy to discern, especially in biological milieu, but it could significantly affect nanospecific material properties, such as homogeneity and colloidal stability, optical and electronic behavior, and cell uptake/targeting properties. As a result, commonly used particle sizing methods are often not conclusive when applied to nanomaterials in complex systems. Additionally, each method possesses its own inherent uncertainties, requiring corroboration of results with one or more additional methods.[99] Alternative strategies such as fluorescence/cellular imaging techniques are being explored to overcome these limitations.[100–102] Therefore, new robust methods must be developed not only to characterize physicochemical properties such as morphology, particle size, polydispersity, and surface charge, but also to assess their *in vivo* performance, such as drug release, metabolism, protein binding, and specific cellular uptake. [96–103]

An additional critical issue in the current regulatory discussions is the increased focus on "nanosimsi- lars." which combine generic drugs and nanocarriers as innovative excipients. Because of the technological complexity of nanoparticulate products, mere bio equivalence studies might not be enough to prove the statistical identity/similarity for nanosimilars, as is the case with generic/biosimilar products.[104,105] The pharmaceutical companies have increased their interest in proof of concept and proof of superiority in clinical efficacy from innovative approaches.[106–108]

Further, the properties of nanomaterials are altered not only by minor changes in raw materials, but also by slight modifications in manufacturing processes. Though limited alterations occur in the structure, the biological properties and the biodistribution patterns might change significantly.[109,110] Thus, another hurdle in the development and clinical translation of nanomaterials has been adaptation of manufacturing processes and scale-up challenges, mainly due to extensive diversity of properties of new materials.[111,112] It is fundamental to identify and control the critical points during each manufacturing process. Applying concepts of quality by design, such as process analytical technologies (PAT), will ensure an on line/at line quality assessment approach.[113,114]

Despite the absence of specific general protocols for preclinical development and characterization for nanomedicines, the regulatory entities from the EU (EMA), USA (FDA), and Japan (PDMA/ MHLW) endeavor to bring about comprehensive and harmonized regulatory propositions in the field of nanomedicines.[115] The Innovative Medicines Initiative (IMI) in Europe and the National Center for Advancing Translational Sciences (NCATS/ NIH) in the United States are both major platforms whose goals include better regulatory sciences in the field of nanomedicines. The Nanotechnology Characterization Laboratory (NCL) at the National Cancer Institute (NCI) in the United States is a major contributor in the development of nanomedicines in oncology.[116]

For Marketing Authorization Applications (MAA) in Europe, the regulatory system allows the opportunity of "scientific counselling" from regulators to applicants, from the early stages of research and development.[117] This can contribute to an increasingly harmonized development of advanced pharmaceuticals and to reduce the impact of major obstacles during their development process. Furthermore, important indicators such as increment in quality-adjusted life years (QALYs) or costs associated to future consecutive hospitalizations must be considered for better pharmacoeconomic evaluation of nanomaterials.[118]

VI. CONCLUSION

To envision the health hazards coupled with engineered nanoparticles, their complete life cycle should be scrutinized from their manufacturing to storage, and from distribution to intended industrial and commercial uses/potential abuse and ultimate disposal. Furthermore, to find ways to manage and confine nanomaterials, we must continue to explore the causes and mechanisms of nanotoxicity to gain better and deeper understanding. Ultimately, a more cautious manipulation of engineered nanomaterials as well as the development of laws and policies for safely managing all aspects of nanomaterial manufacturing, use, and recycling portends the unforeseen opportunities in this blooming field of nanotechnology.

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REFERENCES

1. Bawa R What's in a name? Defining "nano" in the context of drug delivery. Bawa R, editor. Singapore: Pan Stanford Publishing; 2016.

2. Boverhof DR, Bramante CM, Butala JH, Clancy SF, Lafranconi M, West J, Gordon SC. Comparative assessment of nanomaterial definitions and safety evaluation considerations. Regul Toxicol Pharmacol. 2015;73(1):137–50. [PubMed: 26111608]

3. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases. 2007;2(4):MR17–71. [PubMed: 20419892]

4. Raj S, Jose S, Sumod US, Sabitha M. Nanotechnology in cosmetics: opportunities and challenges. J Pharm Bioallied Sci. 2012;4(3):186–93. [PubMed: 22923959]

5. Schäfer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev. 2007;59(6):427–43. [PubMed: 17544165]

6. Katz LM, Dewan K, Bronaugh RL. Nanotechnology in cosmetics. Food Chem Toxicol. 2015;85:127–37. [PubMed: 26159063]

7. Fischman M, Storey E, McCunney RJ, Kosnett M. National Institute for Occupational Safety and Health Nanomaterials and Worker Health Conference—medical surveillance session summary report. J Occup Environ Med. 2011;53(6 Suppl):S35–7. [PubMed: 21606850]

8. Kahru A, Dubourguier HC. From ecotoxicology to nanoecotoxicology. Toxicology. 2010;269(2-3): 105–19. [PubMed: 19732804]

9. Nanowastes Musee N. and the environment: potential new waste management paradigm. Environ Int. 2011;37(1):112–28. [PubMed: 20832119]

10. Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol 2013;3(1–2).

11. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviera N, Warheit D, Yang H; ILSI Research Foundation/

Risk Science Institue Nanomaterial Toxicity Screening Working Group. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol. 2005;2:8. [PubMed: 16209704]

12. Bakand S, Hayes A, Dechsakulthorn F. Nanoparticles: a review of particle toxicology following inhalation exposure. Inhal Toxicol 2012;24(2):125–35. [PubMed: 22260506]

13. Blank MD, Disharoon S, Eissenberg T. Comparison of methods for measurement of smoking behavior: mouthpiece-based computerized devices versus direct observation. Nicotine Tob Res 2009;11(7):896–903. [PubMed: 19525207]

14. Blank F, Gehr P, Rothen-Rutishauser B. In vitro human lung cell culture models to study the toxic potential of nanoparticles In: Sahu SC, Casciano DA, editors. Nanotoxicity: from in vitro and in vivo models to health risks. West Sussex, UK: Wiley; 2007 p. 379–96.

15. Mühlfeld C, Gehr P, Rothen-Rutishauser B. Translocation and cellular entering mechanisms of nanoparticles in the respiratory tract. Swiss Med Wkly. 2008;138(27-28):387–91. [PubMed: 18642134]

16. Asgharian B, Price OT. Airflow distribution in the human lung and its influence on particle deposition. Inhal Toxicol 2006;18(10):795–801. [PubMed: 16774869]

17. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 2004;16(6–7):437–45. [PubMed: 15204759]

18. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect. 2006;114(8):1172–8. [PubMed: 16882521]

19. Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdorster E. The potential risks of nanomaterials: a review carried out for ECE-TOC. Part Fibre Toxicol. 2006;3:11. [PubMed: 16907977]

20. Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, Stone V. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol. 2005;2:10. [PubMed: 16242040]

21. Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, Tran L, Stone V. The pulmonary toxicology of ultrafine particles. J Aerosol Med. 2002;15(2):213–20. [PubMed: 12184871]

22. Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect. 2001;109(Suppl 4):547–51.

23. Löndahl J, Möller W, Pagels JH, Kreyling WG, Swietlicki E, Schmid O. Measurement techniques for respiratory tract deposition of airborne nanoparticles: a critical review. J Aerosol Med Pulm Drug Deliv. 2014;27(4):229–54. [PubMed: 24151837]

24. Ng CT, Yong LQ, Hande MP, Ong CN, Yu LE, Bay BH, Baeg GH. Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and Drosophila melanogaster. Int J Nanomedicine. 2017;12:1621–37. [PubMed: 28280330]

25. Kononenko V, Repar N, Maruši N, Drašler B, Romih T, Ho evar S, Drobne D. Comparative in vitro genotoxicity study of ZnO nanoparticles, ZnO macroparticles and $ZnCl_2$ to MDCK kidney cells: size matters. Toxicol In Vitro. 2017;40:256–63. [PubMed: 28126643]

26. Vinardell MP, Llanas H, Marics L, Mitjans M. In vitro comparative skin irritation induced by nano and non-nano zinc oxide. Nanomaterials (Basel). 2017;7(3):56.

27. Sahu D, Kannan GM, Vijayaraghavan R, Anand T, Khanum F. Nanosized zinc oxide induces toxicity in human lung cells. ISRN Toxicol. 2013;2013:316075. [PubMed: 23997968]

28. Wu J, Liu W, Xue C, Zhou S, Lan F, Bi L, Xu H, Yang X, Zeng FD. Toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicol Lett. 2009;191(1):1–8. [PubMed: 19501137]

29. Sadrieh N, Wokovich AM, Gopee NV, Zheng J, Haines D, Parmiter D, Siitonen PH, Cozart CR, Patri AK, McNeil SE, Howard PC, Doub WH, Buhse LF. Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO_2 particles. Toxicol Sci. 2010;115(1):156–66. [PubMed: 20156837]

30. Crosera M, Prodi A, Mauro M, Pelin M, Florio C, Bellomo F, Adami G, Apostoli P, De Palma G, Bovenzi M, Campanini M. Titanium dioxide nanoparticle penetration into the skin and effects on HaCaT cells. Int J Environ Res Public Health. 2015;12(8):9282–97. [PubMed: 26262634]

31. Hamzeh M, Sunahara GI. In vitro cytotoxicity and genotoxicity studies of titanium dioxide (TiO_2) nanoparticles in Chinese hamster lung fibroblast cells. Toxicol In Vitro. 2013;27(2):864–73. [PubMed: 23274916]

32. Ghosh M, Bandyopadhyay M, Mukherjee A. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: plant and human lymphocytes. Chemosphere. 2010;81(10): 1253–62. [PubMed: 20884039]

33. Mohamed HR, Hussien NA. Genotoxicity studies of titanium dioxide nanoparticles (TiO2NPs) in the brain of mice. Scientifica (Cairo) 2016;2016:6710840. [PubMed: 27034902]

34. Ates M, Demir V, Adiguzel R, Arslan Z. Bioaccumulation, sub-acute toxicity, and tissue distribution of engineered titanium dioxide (TiO_2) nanoparticles in goldfish (Carassius auratus). J Nanomater. 2013;2013:460518. [PubMed: 25383077]

35. Husain M, Wu D, Saber AT, Decan N, Jacobsen NR, Williams A, Vogel U, Halappanavar S. Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. Nanotoxicology. 2015;9(8):1013–22. [PubMed: 25993494]

36. Athinarayanan J, Periasamy VS, Alsaif MA, Al-Warthan AA, Alshatwi AA. Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. Cell Biol Toxicol. 2014;30(2):89–100. [PubMed: 24526451]

37. Fletcher PD, Holt BL. Controlled silanization of silica nanoparticles to stabilize foams, climbing films, and liquid marbles. Langmuir. 2011;27(21):12869–76. [PubMed: 21888342]

38. Dekkers S, Krystek P, Peters RJ, Lankveld DP, Bokkers BG, van Hoeven-Arentzen PH, Bouwmeester H, Oomen AG. Presence and risks of nanosilica in food products. Nanotoxicology. 2011;5(3):393–405. [PubMed: 20868236]

39. Wang P, Zakeeruddin SM, Comte P, Exnar I, Grätzel M. Gelation of ionic liquid-based electrolytes with silica nanoparticles for quasi-solid-state dye-sensitized solar cells. J Am Chem Soc. 2003;125(5):1166–7. [PubMed: 12553808]

40. Yang YX, Song ZM, Cheng B, Xiang K, Chen XX, Liu JH, Cao A, Wang Y, Liu Y, Wang H. Evaluation of the toxicity of food additive silica nanoparticles on gastrointestinal cells. J Appl Toxicol. 2014;34(4):424–35. [PubMed: 24302550]

41. Go MR, Bae SH, Kim HJ, Yu J, Choi SJ. Interactions between food additive silica nanoparticles and food matrices. Front Microbiol. 2017;8:1013. [PubMed: 28638373]

42. Kim IY, Joachim E, Choi H, Kim K. Toxicity of silica nanoparticles depends on size, dose, and cell type. Nanomedicine. 2015;11(6):1407–16. [PubMed: 25819884]

43. Li L, Liu T, Fu C, Tan L, Meng X, Liu H. Biodistribution, excretion, and toxicity of mesoporous silica nanoparticles after oral administration depend on their shape. Nanomedicine. 2015;11(8): 1915–24. [PubMed: 26238077]

44. Huang X, Li L, Liu T, Hao N, Liu H, Chen D, Tang F. The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo. ACS Nano. 2011;5(7): 5390–9. [PubMed: 21634407]

45. Herzog F, Clift MJ, Piccapietra F, Behra R, Schmid O, Petri-Fink A, Rothen-Rutishauser B. Exposure of silver-nanoparticles and silver-ions to lung cells in vitro at the air-liquid interface. Part Fibre Toxicol. 2013;10:11. [PubMed: 23557437]

46. Kokura S, Handa O, Takagi T, Ishikawa T, Naito Y, Yoshikawa T. Silver nanoparticles as a safe preservative for use in cosmetics. Nanomedicine. 2010;6(4):570–4. [PubMed: 20060498]

47. Takamiya AS, Monteiro DR, Bernabé DG, Gorup LF, Camargo ER, Gomes-Filho JE, Oliveira SH, Barbosa DB. In vitro and in vivo toxicity evaluation of colloidal silver nanoparticles used in endodontic treatments. J Endod. 2016;42(6):953–60. [PubMed: 27118468]

48. Hackenberg S, Scherzed A, Kessler M, Hummel S, Technau A, Froelich K, Ginzkey C, Koehler C, Hagen R, Kleinsasser N. Silver nanoparticles: evaluation of DNA damage, toxicity and functional

impairment in human mesenchymal stem cells. Toxicol Lett. 2011;201(1):27–33. [PubMed: 21145381]

49. Kawata K, Osawa M, Okabe S. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol. 2009;43(15): 6046–51. [PubMed: 19731716]

50. Castiglioni S, Caspani C, Cazzaniga A, Maier JA. Short-and long-term effects of silver nanoparticles on human microvascular endothelial cells. World J Biol Chem. 2014;5(4):457–64. [PubMed: 25426268]

51. Rengan AK, Bukhari AB, Pradhan A, Malhotra R, Banerjee R, Srivastava R, De A. In vivo analysis of biodegradable liposome gold nanoparticles as efficient agents for photothermal therapy of cancer. Nano Lett. 2015;15(2):842–8. [PubMed: 25554860]

52. Bastús NG, Sánchez-Tilló E, Pujals S, Farrera C, Kogan MJ, Giralt E, Celada A, Lloberas J, Puntes V. Peptides conjugated to gold nanoparticles induce macrophage activation. Mol Immunol. 2009;46(4):743–8. [PubMed: 18996597]

53. Javed I, Hussain SZ, Shahzad A, Khan JM, Ur-Rehman H, Rehman M, Usman F, Razi MT, Shah MR, Hussain I. Lecithin-gold hybrid nanocarriers as efficient and pH selective vehicles for oral delivery of diacerein—in vitro and in vivo study. Colloids Surf B Biointerf. 2016; 141:1–9.

54. Cheng Y, Dai Q, Morshed RA, Fan X, Wegscheid ML, Wainwright DA, Han Y, Zhang L, Auffinger B, Tobias AL, Rincón E, Thaci B, Ahmed AU, Warnke PC, He C, Lesniak MS. Blood-brain barrier permeable gold nanoparticles: an efficient delivery platform for enhanced malignant glioma therapy and imaging. Small. 2014;10(24):5137–50. [PubMed: 25104165]

55. Ghosh PS, Kim CK, Han G, Forbes NS, Rotello VM. Efficient gene delivery vectors by tuning the surface charge density of amino acid-functionalized gold nanoparticles. ACS Nano. 2008;2(11): 2213–8. [PubMed: 19206385]

56. Villiers C, Freitas H, Couderc R, Villiers MB, Marche P. Analysis of the toxicity of gold nano particles on the immune system: effect on dendritic cell functions. J Nanopart Res. 2010;12(1):55–60. [PubMed: 21841911]

57. Fraga S, Faria H, Soares ME, Duarte JA, Soares L, Pereira E, Costa-Pereira C, Teixeira JP, de Lourdes Bastos M, Carmo H. Influence of the surface coating on the cytotoxicity, genotoxicity and uptake of gold nanoparticles in human HepG2 cells. J Appl Toxicol. 2013;33(10):1111–9. [PubMed: 23529830]

58. Morais T, Soares ME, Duarte JA, Soares L, Maia S, Gomes P, Pereira E, Fraga S, Carmo H, Bastos Mde L. Effect of surface coating on the biodistribution profile of gold nanoparticles in the rat. Eur J Pharm Biopharm. 2012;80(1):185–93. [PubMed: 21946301]

59. Li Y, Kröger M, Liu WK. Shape effect in cellular uptake of PEGylated nanoparticles: comparison between sphere, rod, cube and disk. Nanoscale. 2015;7(40):16631–46. [PubMed: 26204104]

60. Zhang XD, Wu D, Shen X, Liu PX, Yang N, Zhao B, Zhang H, Sun YM, Zhang LA, Fan FY. Size-dependent in vivo toxicity of PEG-coated gold nanoparticles. Int J Nanomed. 2011;6:2071–81.

61. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem 2004;15(4):897–900. [PubMed: 15264879]

62. Gustafson HH, Holt-Casper D, Grainger DW, Ghandehari H. Nanoparticle uptake: the phagocyte problem. Nano Today. 2015;10(4):487–510. [PubMed: 26640510]

63. Mura S, Hillaireau H, Nicolas J, Le Droumaguet B, Gueutin C, Zanna S, Tsapis N, Fattal E. Influence of surface charge on the potential toxicity of PLGA nanoparticles towards Calu-3 cells. Int J Nanomed. 2011;6:2591–605.

64. Grabowski N, Hillaireau H, Vergnaud J, Tsapis N, Pallardy M, Kerdine-Römer S, Fattal E. Surface coating mediates the toxicity of polymeric nanoparticles towards human-like macrophages. Int J Pharm. 2015;482(1–2):75–83. [PubMed: 25448553]

65. Hu YL, Qi W, Han F, Shao JZ, Gao JQ. Toxicity evaluation of biodegradable chitosan nanoparticles using a zebrafish embryo model. Int J Nanomed. 2011;6:3351–9.

66. Eidi H, Joubert O, Attik G, Duval RE, Bottin MC, Hamouia A, Maincent P, Rihn BH. Cytotoxicity assessment of heparin nanoparticles in NR8383 macrophages. Int J Pharm. 2010;396(1–2):156–65. [PubMed: 20542101]

67. Cabeza L, Ortiz R, Prados J, Delgado Á, Martín-Villena MJ, Clares B, Perazzoli G, Entrena JM, Melguizo C, Arias JL. Improved antitumor activity and reduced toxicity of doxorubicin encapsulated in poly(ϵ-caprolactone) nanoparticles in lung and breast cancer treatment: an in vitro and in vivo study. Eur J Pharm Sci. 2017;102:24–34. [PubMed: 28219748]

68. Jang MH, Bae SJ, Lee SK, Lee YJ, Hwang YS. Effect of material properties on stability of silver nanoparticles in water. J Nanosci Nanotechnol. 2014;14(12):9665–9. [PubMed: 25971117]

69. Rocha TL, Gomes T, Sousa VS, Mestre NC, Bebianno MJ. Ecotoxicological impact of engineered nanomaterials in bivalve molluscs: an overview. Mar Environ Res. 2015;111:74–88. [PubMed: 26152602]

70. Chen J, Xiu Z, Lowry GV, Alvarez PJ. Effect of natural organic matter on toxicity and reactivity of nano-scale zero-valent iron. Water Res. 2011;45(5):1995–2001. [PubMed: 21232782]

71. Batley GE, Kirby JK, McLaughlin MJ. Fate and risks of nanomaterials in aquatic and terrestrial environments. Acc Chem Res. 2013;46(3):854–62. [PubMed: 22759090]

72. Biswas P, Wu CY. Nanoparticles and the environment. J Air Waste Manag Assoc. 2005;55(6):708–46. [PubMed: 16022411]

73. Lowry GV, Gregory KB, Apte SC, Lead JR. Transformations of Nanomaterials in the Environment. Environ Sci Technol. 2012;46(13):6893–9. [PubMed: 22582927]

74. Tourinho PS, van Gestel CA, Lofts S, Svendsen C, Soares AM, Loureiro S. Metal-based nanoparticles in soil: fate, behavior, and effects on soil invertebrates. Environ Toxicol Chem. 2012;31(8):1679–92. [PubMed: 22573562]

75. Peralta-Videa JR, Hernandez-Viezcas JA, Zhao L, Diaz BC, Ge Y, Priester JH, Holden PA, Gardea-Torresdey JL. Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. Plant Physiol Biochem. 2014;80:128–35. [PubMed: 24751400]

76. Zhao L, Sun Y, Hernandez-Viezcas JA, Hong J, Majumdar S, Niu G, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL. Monitoring the environmental effects of CeO_2 and ZnO nanoparticles through the life cycle of corn (Zea mays) plants and in situ μ-XRF mapping of nutrients in kernels. Environ Sci Technol. 2015;49(5):2921–8. [PubMed: 25648544]

77. Moghaddasi S, Fotovat A, Khoshgoftarmanesh AH, Karimzadeh F, Khazaei HR, Khorassani R. Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicol Environ Saf. 2017;144:543–51. [PubMed: 28688355]

78. Arts JH, Hadi M, Irfan MA, Keene AM, Kreiling R, Lyon D, Maier M, Michel K, Petry T, Sauer UG, Warheit D, Wiench K, Wohlleben W, Landsiedel R. A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping). Regul Toxicol Pharmacol 2015;71(2 Suppl):S1–27. [PubMed: 25818068]

79. Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, Van Ravenzwaay B, Landsiedel R. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. Toxicol Sci. 2009;112(2):468–81. [PubMed: 19584127]

80. Landsiedel R, Ma-Hock L, Haussmann HJ, van Ravenzwaay B, Kayser M, Wiench K. Inhalation studies for the safety assessment of nanomaterials: status quo and the way forward. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012;4(4):399–413. [PubMed: 22639437]

81. Landsiedel R, Sauer UG, Ma-Hock L, Schnekenburger J, Wiemann M. Pulmonary toxicity of nanomaterials: a critical comparison of published in vitro assays and in vivo inhalation or instillation studies. Nanomedicine (Lond). 2014;9(16):2557–85. [PubMed: 25490426]

82. Landsiedel R, Ma-Hock L, Wiench K, Wohlleben W, Sauer UG. Safety assessment of nanomaterials using an advanced decision-making framework, the DF4nanoGrouping. J Nanopart Res. 2017;19(5):171. [PubMed: 28553159]

83. Eastlake A, Zumwalde R, Geraci C. Can control banding be useful for the safe handling of nanomaterials? A systematic review. J Nanopart Res. 2016;18:169. [PubMed: 27471426]

84. Zalk DM, Nelson DI. History and evolution of control banding: a review. J Occup Environ Hyg. 2008;5(5):330–46. [PubMed: 18350442]

85. Umair M, Javed I, Rehman M, Madni A, Javeed A, Ghafoor A, Ashraf M. Nanotoxicity of inert materials: The case of gold, silver and iron. J Pharm Pharm Sci 2016;19(2):161–80. [PubMed: 27518167]

86. Stefaniak AB, Hackley VA, Roebben G, Ehara K, Hankin S, Postek MT, Lynch I, Fu WE, Linsinger TP, Thünemann AF. Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities. Nanotoxicology. 2013;7(8):1325–37. [PubMed: 23061887]

87. Pisanic TR, Blackwell JD, Shubayev VI, Fiñones RR, Jin S. Nanotoxicity of iron oxide nanoparticle internalization in growing neurons. Biomaterials. 2007;28(16):2572–81. [PubMed: 17320946]

88. Liu G, Gao J, Ai H, Chen X. Applications and potential toxicity of magnetic iron oxide nanoparticles. Small. 2013;9(9-10):1533–45. [PubMed: 23019129]

89. Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of nanoparticles and an overview of current experimental models. Iran Biomed J. 2016;20(1):1–11. [PubMed: 26286636]

90. Tan AS, Berridge MV. Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. J Immunol Methods. 2000;238(1-2):59–68. [PubMed: 10758236]

91. Sayes CM, Warheit DB. Characterization of nanomaterials for toxicity assessment. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009;1(6):660–70. [PubMed: 20049823]

92. Kroll A, Pillukat MH, Hahn D, Schnekenburger J. Current in vitro methods in nanoparticle risk assessment: limitations and challenges. Eur J Pharm Biopharm. 2009;72(2):370–7. [PubMed: 18775492]

93. Raies AB, Bajic VB. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip Rev Comput Mol Sci. 2016;6(2):147–72. [PubMed: 27066112]

94. Valerio LG, Jr. In silico toxicology for the pharmaceutical sciences. Toxicol Appl Pharmacol. 2009;241(3):356–70. [PubMed: 19716836]

95. Deeb O, Goodarzi M. In silico quantitative structure toxicity relationship of chemical compounds: some case studies. Curr Drug Saf. 2012;7(4):289–97. [PubMed: 23062241]

96. Dobrovolskaia MA, McNeil SE. Understanding the correlation between in vitro and in vivo immunotoxicity tests for nanomedicines. J Control Release. 2013;172(2):456–66. [PubMed: 23742883]

97. Gaspar R, Duncan R. Polymeric carriers: preclinical safety and the regulatory implications for design and development of polymer therapeutics. Adv Drug Deliv Rev. 2009;61(13):1220–31. [PubMed: 19682513]

98. Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater. 2009;8(7):543–57. [PubMed: 19525947]

99. Jones CF, Grainger DW. In vitro assessments of nanomaterial toxicity. Adv Drug Deliv Rev. 2009;61(6):438–56. [PubMed: 19383522]

100. Conniot J, Silva JM, Fernandes JG, Silva LC, Gaspar R, Brocchini S, Florindo HF, Barata TS. Cancer immunotherapy: nanodelivery approaches for immune cell targeting and tracking. Front Chem. 2014;2:105. [PubMed: 25505783]

101. Karathanasis E, Suryanarayanan S, Balusu SR, McNeeley K, Sechopoulos I, Karellas A, Annapragada AV, Bellamkonda RV. Imaging nanoprobe for prediction of outcome of nanoparticle chemotherapy by using mammography. Radiology. 2009;250(2):398–406. [PubMed: 19188313]

102. Chapman S, Dobrovolskaia M, Farahani K, Goodwin A, Joshi A, Lee H, Meade T, Pomper M, Ptak K, Rao J, Singh R, Sridher S, Stern S, Wang A, Weaver JB, Woloschak G, Yang L. Nanoparticles for cancer imaging: the good, the bad, and the promise. Nano Today. 2013;8(5):454–60. [PubMed: 25419228]

103. Tinkle S, McNeil SE, Mühlebach S, Bawa R, Borchard G, Barenholz YC, Tamarkin L, Desai N. Nanomedicines: addressing the scientific and regulatory gap. Ann N Y Acad Sci. 2014;1313:35–56. [PubMed: 24673240]

104. Demetzos C, Pippa N. Fractal geometry as a new approach for proving nanosimilarity: a reflection note. Int J Pharm. 2015;483(1-2):1–5. [PubMed: 25659328]

105. Mühlebach S, Borchard G, Yildiz S. Regulatory challenges and approaches to characterize nanomedicines and their follow-on similars. Nanomedicine (Lond). 2015;10(4):659–74. [PubMed: 25723097]

106. Sonnefraud Y, Sinclair HG, Sivan Y, Foreman MR, Dunsby CW, Neil MA, French PM, Maier SA. Experimental proof of concept of nanoparticle-assisted STED. Nano Lett. 2014;14(8):4449–53. [PubMed: 25053232]

107. Sheridan C Proof of concept for next-generation nanoparticle drugs in humans. Nat Biotechnol. 2012;30(6):471–3. [PubMed: 22678364]

108. Lesaffre E, Superiority, equivalence, and non-inferiority trials. Bull NYU Hosp Jt Dis. 2008;66(2):150–4. [PubMed: 18537788]

109. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng. 2012;14:1–16. [PubMed: 22524388]

110. Castranova V Overview of current toxicological knowledge of engineered nanoparticles. J Occup Environ Med 2011;53(6 Suppl):S14–7. [PubMed: 21606847]

111. Geraci C, Heidel D, Sayes C, Hodson L, Schulte P, Eastlake A, Brenner S. Perspectives on the design of safer nanomaterials and manufacturing processes. J Nanopart Res. 2015;17(9):366. [PubMed: 26435688]

112. Paliwal R, Babu RJ, Palakurthi S. Nanomedicine scale-up technologies: feasibilities and challenges. AAPS PharmSciTech 2014;15(6):1527–34. [PubMed: 25047256]

113. Li J, Qiao Y, Wu Z. Nanosystem trends in drug delivery using quality-by-design concept. J Control Release. 2017;256:9–18. [PubMed: 28414149]

114. Rathore AS. Roadmap for implementation of quality by design (QbD) for biotechnology products. Trends Biotechnol 2009;27(9):546–53. [PubMed: 19647883]

115. Wagner V, Dullaart A, Bock AK, Zweck A. The emerging nanomedicine landscape. Nat Biotechnol. 2006;24(10):1211–7. [PubMed: 17033654]

116. Gaspar R Nanoparticles: pushed off target with proteins. Nat Nanotechnol. 2013;8(2):79–80. [PubMed: 23380930]

117. Sainz V, Conniot J, Matos AI, Peres C, Zupancic E, Moura L, Silva LC, Florindo HF, Gaspar RS. Regulatory aspects on nanomedicines. Biochem Biophys Res Commun. 2015;468(3):504–10. [PubMed: 26260323]

118. Benedict A, Cameron DA, Corson H, Jones SE. An economic evaluation of docetaxel and paclitaxel regimens in metastatic breast cancer in the UK. Pharmacoeconomics. 2009;27(10): 847–59. [PubMed: 19803539]

119. Beddoes CM, Case CP, Briscoe WH. Understanding nanoparticle cellular entry: a physicochemical perspective. Adv Colloid Interf Sci. 2015;218:48–68.

120. Winkler DA, Mombelli E, Pietroiusti A, Tran L, Worth A, Fadeel B, McCall MJ. Applying quantitative structure-activity relationship approaches to nanotoxicology: current status and future potential. Toxicology. 2013;313(1):15–23. [PubMed: 23165187]

121. Chouly C, Pouliquen D, Lucet I, Jeune JJ, Jallet P. Development of superparamagnetic nanoparticles for MRI: effect of particle size, charge and surface nature on biodistribution. J Microencapsul 1996;13(3):245–55. [PubMed: 8860681]

122. Mansouri E, Khorsandi L, Orazizadeh M, Jozi Z. Dose-dependent hepatotoxity effects of zinc oxide nanoparticles. Nanomed J. 2015;2(4):273–82.

123. Dubey A, Goswami M, Yadav K, Chaudhary D. Oxidative Stress and Nano-Toxicity Induced by TiO_2 and ZnO on WAG Cell Line. PLoS One. 2015;10(5):e0127493. [PubMed: 26011447]

124. Luo YH, Chang LW, Lin P. Metal-based nanoparticles and the immune system: activation, inflammation, and potential applications. Biomed Res Int 2015;2015:143720. [PubMed: 26125021]

125. Han L, Su L, Chen D, Zhang S, Zhang Y, Zhao B, Miao J. ZnS nanoarchitectures induced dysfunction of vascular endothelial cells in vitro and in vivo. Environ Toxicol. 2015;30(7):755–68. [PubMed: 24449189]

126. Durazo SA, Kompella UB. Functionalized nanosystems for targeted mitochondrial delivery. Mitochondrion. 2012;12(2):190–201. [PubMed: 22138492]

127. Wan R, Mo Y, Feng L, Chien S, Tollerud DJ, Zhang Q. DNA damage caused by metal nanoparticles: involvement of oxidative stress and activation of ATM. Chem Res Toxicol. 2012;25(7):1402–11. [PubMed: 22559321]

128. Prietl B, Meindl C, Roblegg E, Pieber TR, Lanzer G, Fröhlich E. Nano-sized and micro-sized polystyrene particles affect phagocyte function. Cell Biol Toxicol. 2014;30(1):1–16. [PubMed: 24292270]

129. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K. Silica nanoparticles as hepatotoxicants. Eur J Pharm Biopharm. 2009;72(3):496–501. [PubMed: 19232391]

| Chantecaille Nano Gold Energizing Cream | Trucare Nano Silver Toothpaste Anti Bacterial, Fights Ulcers Canker Sore | Melaklear Nano Alpha Arbutin Anti Melasma Spots SPF20 Skin Lightening Cream |
| Research In Beauty Nano-Complex Keratin Gold Shampoo | Acz Nano Zeolite Extra Strength-Detoxification Supplement | Cyclic Nano Silver Cleanser Soap |

FIG. 1:
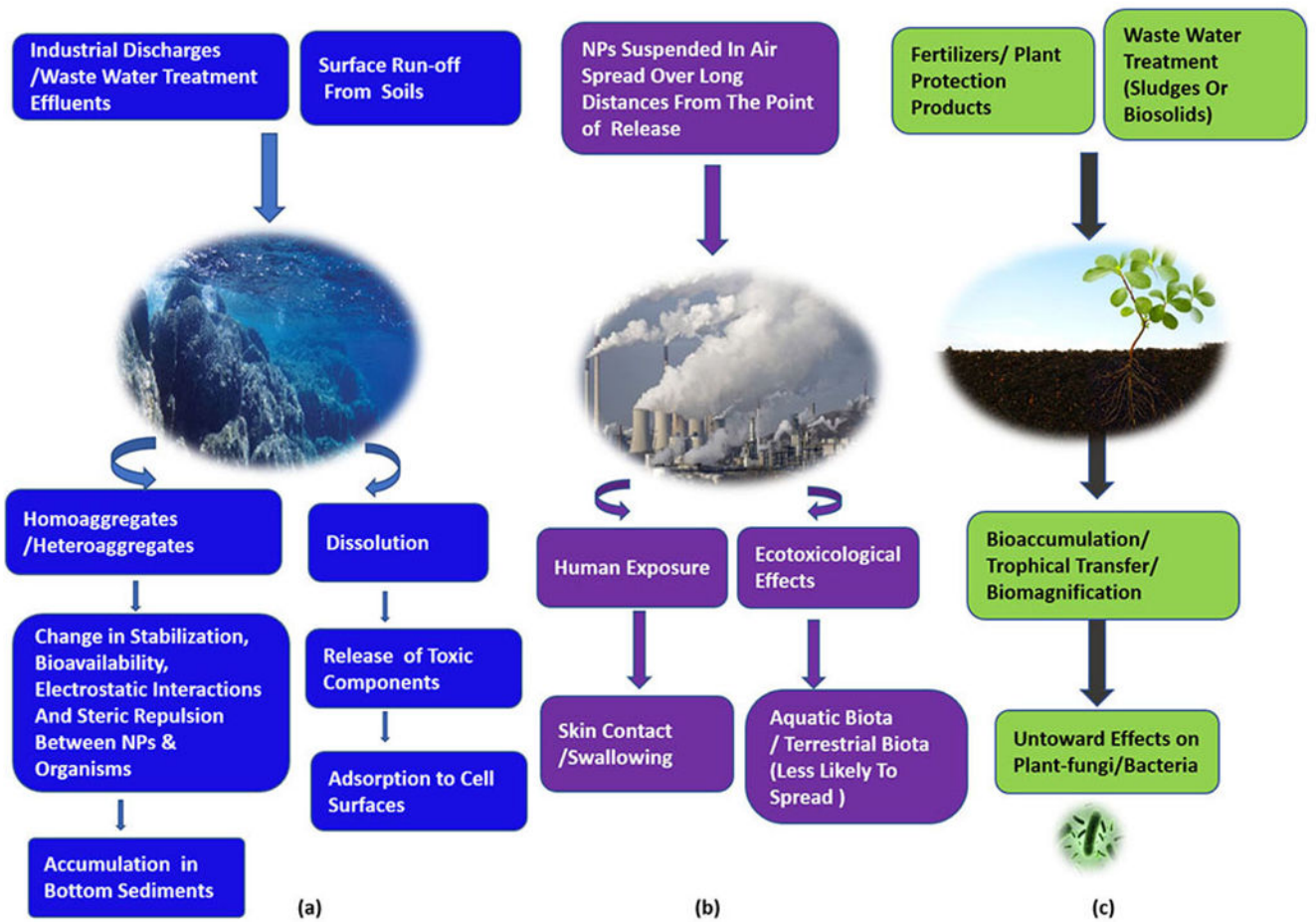Examples of engineered nanomaterials in daily life

FIG. 2:
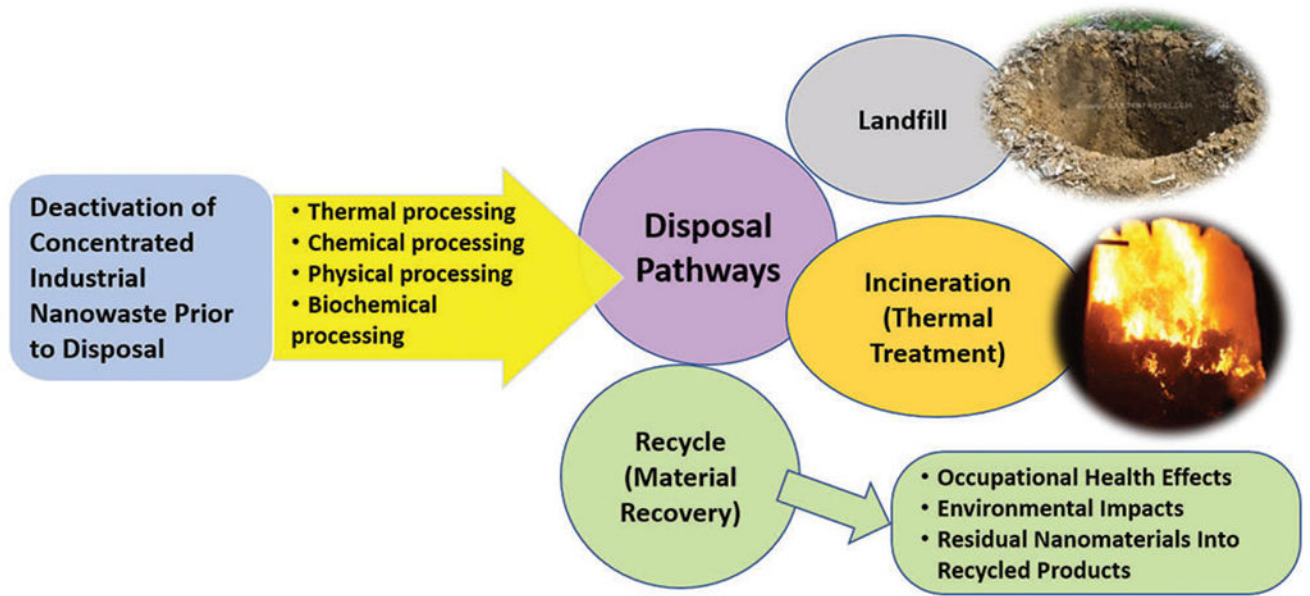Engineered nanoparticles pathways in different environments: (a) in water; (b) in air; (c) in soil

FIG. 3:
Engineered nanoparticles disposal pathways

TABLE 1:

Mechanisms of engineered nanoparticle toxicity

Mechanisms of toxicity		Reference number
Cellular uptake	Direct intracellular entry	119
	Cell membrane binding	120
	Uptake through reticuloendothelial system	121
Catalytic activity	Release of more reactive ionic fonn from nanoparticle surface	60
	ROS generation, oxidative stress	24,122
	Lipid peroxidation	32, 34
	Protein denaturation	123
	Inflammation	35, 124
	Endothelial dysfunction	125
	Mitochondrial perturbation	126
Genotoxicity	DNA damage, mutations	33, 48, 127
Cellular dysfunction	Phagocytic function impairment	128
	Altered cell cycle regulation	36

TABLE 2:

Various experimental models used to demonstrate nanoparticle toxicity.

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
ZnONP	50 nm and 100 nm	HaCaT keratinocytes, 3D-epidermis	• Reduced cell viability of HaCaT keratinocytes in dose-dependent and time-dependent manner • No irritation in 3D-epidermis model at 1 mg/mL after 24 h of exposure	26
ZnONP	70 nm; 0–10 mg/mL	Human lung fibroblast MRC5 cells (*in vitro*) Drosophila larvae (*in vivo*)	• Gut cells of Drosophila larvae showed a dramatic increase in the ROS levels, leading to a decrease in viability *in vivo* • MRC5 cells lost their membrane integrity and released the LDH in a dose-dependent manner • Cell viability was significantly decreased as early as 24 h after treatment, and ZnO NPs at a concentration of 50 μg/mL (617 μM) caused total cell death • Significant decrease in total DNA content of treated cells at both S and G2/M phases of cell cycle • ROS production lead to accumulation of 8-OHdG, an oxidized DNA nucleoside indicating oxidative DNA damage	24
ZnONP	72 nm; 61, 123 μM	Madin-Darby canine kidney (MDCK) cells	• Size-dependant genotoxic effect was demonstrated using MMS solution (0.45 μM) as a positive control • A statistically significant increase in double- and single-strand DNA breaks in cells at sub-cytotoxic concentrations accompanied by a reduction of CAT and GST activity	25
ZnONP	Below 100 nm; 5, 50, and 300 mg/kg for 14 days	Rats (oral)	• 5 mg/kg dose induced significant increase in oxidative stress through increase in MDA content and decrease in SOD and GPx enzymes activity in the liver, and elevation in plasma AST, ALT, and ALP levels • 5 mg/kg of ZnONPs caused hepatocytes swelling, congestion of RBC, accumulation of inflammatory cells and significantly increased apoptotic index • Dose of 300 mg/kg had poor hepatotoxicity effect	122
ZnONP	50 nm; 5, 25, 50, and 100 μg/mL for 24 h	Human lung epithelial cells (L-132)	• Depletion of GSH level and an increase in ROS levels suggested incidence of oxidative stress • DNA fragmentation (apoptotic cell death) • Expression of metallothionein gene, touted as a biomarker for metal-induced toxicity	27

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results		Reference number
TiO₂NP	Less than 50 nm; 500 mg/kg; for 5 consecutive days & sacrificed after 24 h, 7 days, or 14 days	Mice (oral)	•	Animals showed mild to moderate changes in the cytoarchitecture of brain tissue in a time dependent manner.	33
			•	Comet assay revealed apoptotic DNA fragmentation.	
			•	Point mutation of presenilin 1, gene linked to Alzheimer's disease (PCR-SSCP and direct sequencing).	
TiO₂NP	10-30 nm; 10 and 100 mg/kg	Goldfish (*Carassius auratus*)	•	Accumulation of TiO₂NP increased from 42.71 to 110.68 ppb in the intestine and from 4.10 to 9.86 ppb in the gills with increasing exposure dose from 10 to 100 mg/L TiO₂NP.	34
			•	MDA, a biomarker of lipid oxidation was detected in the liver of goldfish.	
			•	TiO₂NP exposure also inhibited growth of goldfish.	
TiO₂NP	21 nm; 6.0–10 mg/m³	Female C57BL/6 mice (intratracheal instillation)	•	ELISA analysis revealed activation of complement cascade and inflammatory processes in heart and specific activation of complement factor 3 in blood.	35
TiO₂NP	1–200 nm; uncoated (anatase and rutile); polyacrylate-coated; 10 and 100 mg/L for 24 h	Chinese hamster lung fibroblast (V79) cells	•	Both coated and uncoated TiO₂NP decreased the cell viability in a mass- and size-dependent manner. Polyacrylate coated particles were only cytotoxic at high concentration (100 mg/L).	31
			•	DNA damage was displayed only by uncoated TiO₂NP, and a more genotoxicity effect was observed in nano-sized anatase compared to rutile.	
TiO₂NP	154 nm;1.0 g/L; 24 h, 48 h, 7 days	Human HaCaT keratinocytes	•	TiO₂NP did not permeate intact/damaged skin and exerted a low cytotoxicity effect only at a high dose and long exposure.	30
TiO₂NP	20–500 nm; 5%; uncoated and coated particles	Skin of Yucatan mini-pigs	•	TiO₂NP from sunscreen formulations did not penetrate the intact epidermis.	29
TiO₂NP	4 nm, 60 nm; 5%; 30, 60 days	Pig ear, hairless mice (topical)	•	30 days after topical application on pig ear, TiO₂NP penetrated into deep layer of epidermis.	28
			•	*In vivo* experiments with hairless mice showed penetration of particles in various tissues such as brain (10-15 µg/g) lung (12–18 µg/g), and spleen (22–30 µg/g) after 60 days of exposure.	
TiO₂NP	100 nm; (0, 2, 4, 6, 8, and 10 mM) (0, 0.25, 0.50, 0.75, 1, 1.25,1.50, 1.75, 2 mM)	*Allium cepa*: human lymphocytes	•	Increased MDA concentration at 4 mM treatment dose in *Allium cepa* indicated lipid peroxidation as possible mechanism for DNA damage	32, 55

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
			• Genotoxicity in human lymphocytes was shown at a low dose of 0.25 mM followed by a decrease in extent of DNA damage at higher concentrations	
SiO_2NP	Short rods ARs of ~1.5; length of 185 nm (22 nm) and long rods (ARs of ~5, length of 720 nm (65 nm)	Mice (intravenous injection)	• Short-rod SiO_2NP trapped in liver, while long-rods distributed in spleen	44
			• Short-rods showed a more rapid clearance rate than long-rods in both urine and feces	
			• MSNs with both aspect ratios were present in higher concentration in lungs after PEG modification	
			• Hematology, serum biochemistry, and histopathology results indicated no significant toxicity *in vivo*	
SiO_2NP	Spherical SiO_2NP AR of 1, diameter of 83 nm; short-rods AR of 1.75, length of 146 nm (83 nm); long-rods AR of 5, length of 483 nm (96 nm); 40 mg/kg 100 µL.	Male mice (oral)	• Unlike intravenous administration, there were no intact SiO_2NP (AR = 1, 1.75, and 5) in urine at 24 h post oral administration	43
			• Most of the orally administrated SiO_2NP were rapidly excreted from feces, but some intact SiO_2NP or their degradation products were absorbed through intestinal mucosa, entered into systematic circulation, and finally excreted via renal excretion	
			• With the decrease of aspect ratio, the systematic absorption by small intestine and other organs increased and the urinary excretion decreased	
			• Renal tubular necrosis, hemorrhage and vascular congestion in the renal interstitium were observed for all three SiO_2NP irrespective of the geometrical features, suggesting possible kidney damage	
			• All three SiO_2NP showed no obvious hematological toxicity	
SiO_2NP	20–200 nm; 10–500 µg/mL	Three different cell types A549 and HepG2 epithelial cells and NIH/3T3 fibroblasts	• *A549 cells*: size and dose-independent toxicity for doses 50 µg/mL for all but the 60 nm SiO_2NP after 72 h of exposure. For the 60 nm particles, high doses (200 and 500 µg/mL) resulted in drastically reduced viability in comparison to all other SiO_2NP doses and sizes tested ($p > 0.05$)	42
			• *NIH/3T3 cells*: size and dose dependent reductions in viability at both 24 and 72 h of exposure to SiO_2NP at doses 50 (µg/ml (500 µg/ml). 60 nm SiO_2NP caused an extreme reduction in NIH/3T3 cell viability to 4.55 ± 1.75% and 1.30 ± 0.26% of control after 24 and 72 h of exposure, respectively	
			• *HepG2 cells*: no size dependent changes in viability in response to SiO_2NP doses greater than 50 µg/mL, except for the highest dose of 60 nm SiO_2NP which caused a significantly	

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
			greater reduction in viability than any other SiO$_2$NP treatment ($p > 0.05$)	
SiO$_2$NP	200–460 nm; four different SiO$_2$NP food additives	Gastrointestinal cell lines GES-1 and human colorectal adenocarcinoma cell Caco-2; (*in vitro* gastrointestinal toxicology)	• At low concentration (100 µg-mL^{-1} and less) no adverse effect shown by gastrointestinal cell lines, even after 72 h exposure making them safe to be used as food additive • At higher concentrations of 200 µg/ml or more a significant change in cell functions after 48-h exposure • SiO$_2$NP did not change the cell morphology or induce apoptosis/necrosis, they rather entered the cells and inhibited the cell growth by cell cycle arrest	40
SiO$_2$NP	24 nm	Interactions between SiO$_2$NP and surrounding food matrices	• Concentration, but not time-dependent interactions between SiO$_2$NPs and saccharides were found, and these interactions significantly increased when incubation temperature increased at 40°C • Comparable interactions between casein and NPs at 25°C and at 40°C, except at 7 d postincubation • Significantly reduced fluorescence quenching at 4°C. Blue shift observed only at 40°C after 48 h of incubation • < 1.8% oleic acid and 0.4% palmitic and linoleic acids in olive oil interacted with SiO$_2$NP, and the effects of incubation time and temperature on the interactions were not observed ($p > 0.05$)	41
SiO$_2$NP	70, 300, and 1000 nm; 10, 30, 100 mg/kg doses	BALB/c male mice (intravenous injection)	• 70 nm NP-induced liver injury at 30 mg/kg dose, while 300 nm or 1000 nm NP shown no effect even at 100 mg/kg dose • 70 nm NP increased serum markers of liver injury, serum aminotransferase and inflammatory cytokines in dose-dependent manner • Repeated administration of 70 nm NP twice a week for 4 weeks, even at 10 mg/kg, caused hepatic fibrosis	129
AgNP	730 nm; 0.2% to 2%	Human skin; HaCaT keratinocytes	• No cytotoxicity against keratinocytes and no effect on UVB-induced cell death demonstrated the potential of AgNP for use as a preservative in cosmetics at these levels • However, AgNP could penetrate the disrupted human skin (0.002–0.02 ppm)	46
AgNP	7–10 nm; stabilized with polyethylenimine	Human hepatoma cell line, HepG2	• Nanosized Ag particles as well as "ionic Ag^{+}" contributed to the toxic effects • AgNPs caused much stronger damages to chromosome than polystyrene NPs and ionic Ag$^+$	49

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
AgNP	Less than 50 nm; 0.1, 10 µg/ml; 1, 3, and 24 h	Human mesenchymal stem cells (hMSCs)	• Cytotoxicity besides abnormal changes in cell morphology and cellular shrinkage at higher doses (> 1.0 mg/L) • Cytotoxicity and genotoxicity were demonstrated in hMSCs at significantly higher concentrations as compared to antimicrobial effective levels • Cytotoxic effects were observed at concentrations of 10 µg/mL for all test exposure periods • Comet assay and the chromosomal aberration test showed DNA damage after 1, 3, and 24 h at 0.1 µg/mL	48
AgNP	35 nm; 0.1, 0.5, 1.0, 2.5 and 5 µg/mL	Human microvascular endothelial cells (HMEC); endothelial colony-forming cells (ECFC)	• Dose dependent viability reduction of HMEC after 72 h. The inhibitory concentration 50 (IC_{50}) was 1.86 ± 0.13 (µg/mL, and statistically significant cytotoxicity was observed at a concentration 0.1 µg/mL ($P < 0.01$) • 24 h treatment of ECFC by AgNP (2.5 and 5 µg/mL) induced a dose dependent but reversible genotoxic stress in comet assay	50
AgNP	5 nm; 0.1–100 µg/mL; stabilized with ammonia (SNA) or PVP (SNP)	Mouse fibroblast L929; Wistar albino rats (implant of polyethylene tubes filled with a fibrin sponge embedded in 100 mL SNA or SNP)	• SNA and SNP did not show cytotoxicity to cell line L929 at 25 µg/mL or lower concentrations • SNA and SNP were cytotoxic to L929 in higher concentrations, with SNA significantly more toxic than SNP • In rats, a moderate inflammatory response consisting mainly of neutrophils was observed 7 days after the implantation of polyethylene tubes and this inflammatory response was reduced with time	47
AuNP	2 nm; quaternary ammonium functionalized AuNP and carboxylate-substituted AuNP	Red blood cells, Cos-1 cells, and bacteria (*E. coli*).	• Cationic (quaternary ammonium functionalized) AuNP were moderately toxic, whereas anionic (carboxylate-substituted) AuNP were nontoxic • Concentration-dependent cell-lysis mediated by initial electrostatic binding was observed	61
AuNP	5, 10, 30, and 60 nm; 4000 (µg/kg; 200 µL; PEG-coated	Mice (intraperitoneal injection)	• Size-dependent accumulation of PEG-coated AuNP in different organs • *In vivo* toxicity was not size-dependent. The toxicity of 10 nm and 60 nm particles was found to be higher than that of 5 nm and 30 nm particles • Significant increase in ALT and AST levels caused by 10 nm and 60 nm AuNPs, indicated liver damage	60

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
AuNP	18.4 ± 4.9 nm; 0.6–1 mg Au/kg coatings: citrate, 11-MUA and 3 pentapeptides, CALNN, CALND, and CALNS	Rats (intravenous injection)	• Similar biodistribution profiles for citrate and MUA-coated AuNPs at 24 h postinjection. Whereas, significantly larger levels of peptide-capped AuNPs observed in liver (74%-86%) • Peptide capping significantly increased hepatic uptake, showing the influence of AuNPs functionalization in biodistribution	58
AuNP	Different shaped PEGylated AuNP (cubic, rod, disk, star) with identical surface area and grafting density 1.6 chains per nm^2	Dissipative particle dynamics (DPD; molecular simulation model)	• Spherical AuNP exhibited the fastest internalization rate, followed by cubic, rodlike, and then disklike particles • For AuNP with high aspect ratios (rod and disk) the internalization rates were highly dependent on the entry angle, and delayed by membrane deformation and complicated rotation of these AuNP • Star-shaped particles were quickly wrapped by the cell membrane, indicating their potential for drug delivery	59
AuNP	20 nm; (200 μM Au); citrate (cit) and 11-mercaptoundecanoic acid (11-MUA) coatings	Human liver HepG2 cells	• Both AuNP were internalized in a concentration-dependent manner but without a significant difference in their extent of internalization • Neither of two differently coated AuNP induced significant cytotoxicity • In spite of absence of cytotoxicity, cit-AuNP exhibited DNA damage at lower concentrations.	57
AuNP	10 nm; (13 mM Au)	Dendritic cells (extracted from bone marrow of C57BL/6 mice)	• AuNP were not cytotoxic for dendritic cells • But these AuNP reduced the secretion of IL12p70 when the cells were simultaneously activated by addition of lipopolysaccharides (LPS)	56
PNP	100 nm, and 200 nm; PLGA-NP coated with CS, PF68 and PVA	Human bronchial Calu-3 cells	• NP were internalized by Calu-3 cells and they induced low toxicity even at high concentrations, independent of their surface chemistry and charge	63
PNP	200 nm; PLGA-NP coated with CS, PF68 and PVA; 0.1,1 mg/mL	Humanlike THP-1 macrophages	• Stabilizer-free PLGA NP exerted no cytotoxicity • The stabilizers alone also didn't confer toxicity to NP. • No or scarce signs of toxicity was shown at therapeutic concentrations (up to 0.1 mg/mL) in vitro • At high concentrations (above 1 mg/mL), significant cytotoxicity was found to be induced by the presence of all stabilizers	64
PNP	CS NP; 30 mg/L (200 nm) and 40 mg/L (340 nm)	Zebrafish embryos	• CS NP induced malformations, including a bent spine, pericardial edema, and an opaque yolk in zebrafish embryos	65

Nanoparticles	Size/dose/exposure time	Experimental model/route	Results	Reference number
			• They increased rate of cell death, high expression of reactive oxygen species, as well as overexpression of heat shock protein 70, indicating physiological stress in zebrafish	
PNP	~80 nm; doxorubicin-loaded PLC NP	Breast and lung cancer cell lines from both humans and mice	• DOX-loaded PCL NP increased the drug internalization in both breast and lung tumor cells • Moreover, DOX-loaded PCL NP were able to reduce DOX toxicity *in vivo* without causing myocardial injury or modulation of blood cell parameters	67
PNP	54 to 400 nm; LMWH encapsulated in Eudragit RS and PCL using nanoprecipitation and double emulsion methods	NR8383 macrophages	• The bioavailability of LMWH was increased by polymer encapsulation. Encapsulation dramatically reduced the cationic NP toxicity owing to the serious modifications in particle zeta potential • The preparation method as well as the assay conditions influenced the encapsulation efficiency and cytotoxic effect profiles • DE/RS+ and DE/PCL+ were considered as a satisfactory nanosized delivery system for LMWH because of their high encapsulation efficiency and low toxicity	66

[a] 11-MUA:11-mercaptoundecanoic acid; ALP: alkaline phosphatase; ALT: alanine aminotransferase; ARs: aspect ratios; AST: aspartate aminotransferase; CALND: Cys-Ala-Leu-Asn-Asp; CALNN: Cys-Ala-Leu-Asn-Asn; CALNS: Cys-Ala-Leu-Asn-Ser; CAT: catalase; CS: chitosan; DE: double emulsion; DOX: doxorubicin; GPx: glutathione peroxidase; GSH: glutathione; GST: glutathione S-transferase; IL-β: interleukin-1β; LDH: lactate dehydrogenase; LMWH: low molecular weight heparin; LPS: lipo-poly-saccharides; MDA: malondialdehyde; MMS: methyl methanesulfonate; NP: nanoprecipitation;

PCL: poly(ε-caprolactone); PEG: polyethylene glycol; PF68: poloxamer 188; PLGA: poly(lactic-co-glycolic acid); PVA: poly(vinyl alcohol); RS: eudragit® RS; SOD: superoxide dismutase; TNF–α: tumor necrosis factor alpha.